Gross And Histopathological Lesion In The Gills Of Naturally Infected Common Barb *Barbodes binotatus* by *Dactylogyrus*

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Abstract
This research was conducted to observe gills-naturally infected common barb *Barbodes binotatus* by *Dactylogyrus* and the changes it caused both gross and histopathological changes. As many as 40 samples of common barb was found with different degree of *Dactylogyrus*. Gill discoloration, swelling, excess mucus, and structural damage were found on the gills of infected common barb, while hyperplasia, fusion, vacuoles and telangiectasia found in the histopathological observation. This study also conducted a histopathological assessment with a scoring value. As a record of *Dactylogyrus* infestation on common barbs, this research encourages awareness of the gill damage to the tissue level caused by this parasite.

Key word : Common barb, *Dactylogyrus*, histopathology, gross lession.

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Introduction

Common barb is an endemic fish in Indonesia, become one of the consumption fish with high economic value. This fish is usually sold at a price of IDR 30,000 - 40,000/kg (Zubaidah *et al*., 2021). With this high price, common barb has begun to be cultivated by several local farmers in Indonesia. Several reports of *Dactylogyrus* infestations in common barb, however, have been reported (Wahab et al., 2021; Mohanta and Chandra, 2000), but in Indonesia, the report of *Dactylogyrus* infestation in common barb was still less.

Gills are organs in fish that have a vital function in the process of respiration. Changes in the gills due to parasitic infestation can certainly have a negative impact on aquaculture activities. Unfortunately, observing ectoparasites on gills is not easy to do due to the small size of the parasite and its hidden location between the gill lamellae. One way that can be done to observe parasitic infestations on the gills is through examining changes in the anatomy of the gills. The existence of ectoparasitic infestation on the gills itself can not directly be used to certain that the appearance of symptoms comes from the parasitic disease. In this research, we observed the pathological changes, either gross or histopathological lesion to give a description of the damage level caused by *Dactylogyrus* to the gills.

Materials and Methods

Study site information and fish colletion

As much as 40 common barb from the fish farmers in Pasuruan, East Java (Figure 1A), were used in this study between December, 2022 to Januari 2023. The size of the fish used were 9-20 cm and 11-15 gram in weight (Figure 1B).

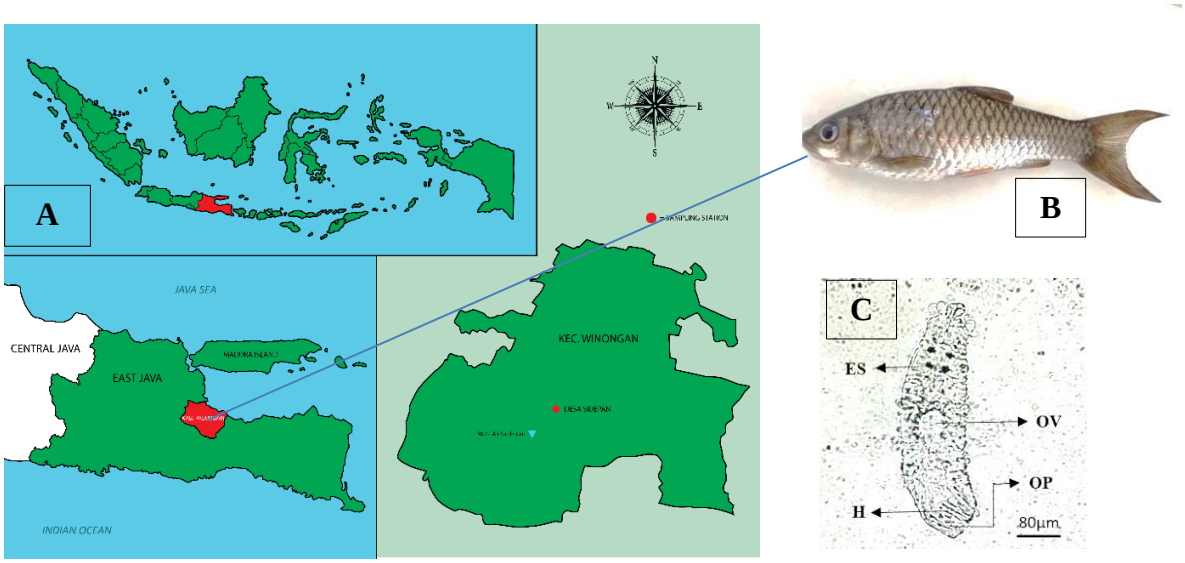

Figure 1. Host and parasite information. A. geographical of sampling localities. B. Common carp. C. *Dactylogyrus*, (ES) eyespot, (OV) ovarium, (H) hook, (OP) ophisthaptor (100x)

Table 1: Study Site Information

Geographical location	Elevation ±40 mpl and ±25 km from the coastline
Water temperature	24 – 25 °C
Air temperature	25 – 30 °C
Ponds	*Indoor* and *Outdoor*
Water source	Upflow umbulan (±5000 l/s)
Electric power	220 W
Waterways	50 m
Average water discharge	120 l/s

Dactylogyrus examination

Dactylogyrus were recovered from gill filaments of common barb according to Kumar (2021) and Manurung and Gaghenggang, 2016). The examination begins with taking the fish gills, then scraped by razor and smeared on the object glass. The smears were observed under a microscope with 100x magnification. The number of *Dactylogyrus* was counted using a hand counter. *Dactylogyrus* was observed based on the presence of attachment apparatus and copulatory organ (Zolovs and Kurjusina, 2012).

Determination of the degree of infestation of Dactylogyrus

Determination of the degree of infestation was done based on the intensity value of *Dactylogyrus*. The degree of infestation of *Dactylogyrus* in this study was determined based on Sarimudin et al., (2016). Low degree of infestation was determined when the intensity value ranges from 1-5 ind/fish; moderate degree of infestation was determined when the intensity value ranges from 6-10 ind/fish; and severe degree of infestation was determined when the intensity value more than 10 ind/fish.

Intensity was calculated based on formula Bush et al., (1997) and Rustikawati et al., (2004) :

$$\text{Intensity} = \frac{\text{number of parasite found}}{\text{number of fish infected by parasite}}$$

Gross and histopathological lession of gills infected by Dactylogyrus

Gross lession of gills infected by *Dactylogyrus* were observed visually for their structure, color, and mucous production. All the gross lession of gills were documented. The gills from the infected fish were also used to observe the histopathological change under light microscope. Gills were fixed ini neutral buffered formalin 10% and 3-μ thick sections were made in paraffin which stained with hematoxylin-eosin (H&E). The procedure for histopathological preparation were modified from Wulansari et al 2020 and Suliman et al., 2021. Histopathology observation were done under light-microscope with 400x magnification and the cell damage were scored according to Corley *et al*., 2013 and Yanuhar et al., 2020 with the formula :

$$\text{Damage Percentage} = \frac{\text{total damage cell}}{\text{total analysed cell}} \text{ x } 100\%$$

The damage percentage then scored from number 1 to 4. Number 1 (mild) has a percentage level of tissue damage of less than 25%. Number 2 (moderate) has a percentage level of tissue damage of 26-50%. Number 3 (severe) has a percentage level of tissue damage of 51-75%. Number 4 (very heavy) has a percentage level of tissue damage of 76-100%. All necropsy and tissue sampling procedure were carried out under aseptic conditions with aseptic surgical instruments. Furthermore, gross lesion and histopathological changes were analyzed descriptively (Putra et al., 2017).

Results

The results showed that as many as 40 samples were positively infected by *Dactylogyrus* on gill organ. *Dactylogyrus* found with morphological characteristics of a flattened body shape, having two pairs of eyespots, an ovary, a hook or hook, four protrusions on the anterior and an ophisthaptor on the posterior (Figure 1C). This study also showed variations in the degree of infestation of *Dactylogyrus* in common barb fish. Low, moderate, and severe degree of infestation of *Dactylogyrus* were recorded in Common barb. From the total number of samples, low degree of was the most commonly found in this study with 2.29 in intensity (Table 2).

Table 2. The results of the examination of *Dactylogyrus* from common barb

Degree of Infestation (Sarimudin, 2016)	**Number of *Dactylogyrus* found**	**Number of fish infected**	**Intensity (ind/fish)**
Low	48	21	2.29
Moderate	84	13	6.46

Severe	80	6	13.33

Histopathological observations showed tissue damage occurs in low, moderate, and severe degree of infestation in the form of hyperplasia, secondary lamella fusion, vacuoles, and telangiectasia (Figure 2). The level of gill tissue damage caused by *Dactylogyrus* infestation showed a score of 1 to 3 (Table 3).

Table 3. Histopathological examination of the gills of common barbs

Fish sample	**Number of fish examined**	**Degree of infestation**	**Histopathological changes**	**Scoring (Mean ± SD)**
Common barb	40	Mild	- Hyperplasia	1,02 ± 0,89
		Moderate	- Hyperplasia - Secondary lamella fusion	2,04 ± 1,13
		Severe	- Hyperplasia - Secondary lamella fusion - Vacuola - Telangiectasia	3,06 ± 1,19

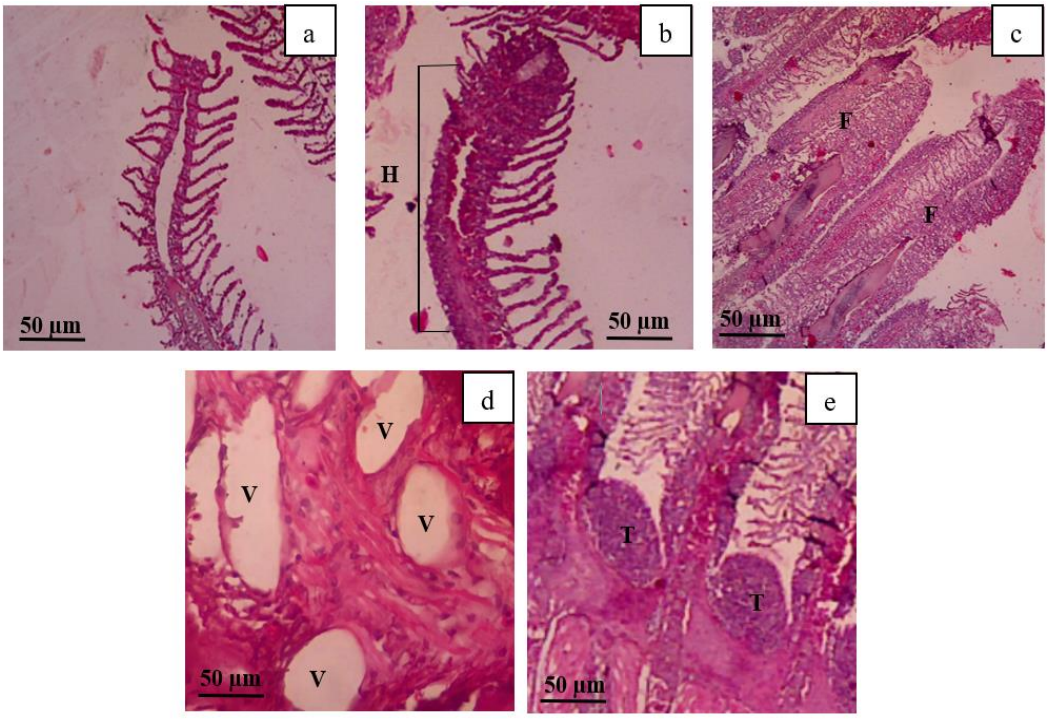

Figure 2. Histopathology of the gills of *Dactylogyrus* infested common barbs under 400x microscope magnification. Description: (a) Normal, (b) Hyperplasia (H), (c) Secondary lamella fusion (F), (d) Vacuoles (V), (e) Telangiectasia (T).

Discussion

The variation in the degree of *Dactylogyrus* infestation found in this study could be affected by the environment, species and age of fish, fish genetics, vectors, nutrition, and fish activity (Hardi, 2015). Generally, fish with healthy condition are able to control the number of parasites in their bodies (Kumar et al., 2021). This could be the reason that the most commonly found in this study is low degree of infestation.

Gross lesion of gill found in this study were showed by the appearance of some color change, over production of mucus which fill the gill area, and the gill-unneatly structured. The most heavy gross lesion were found in the severe degree of infestation which showed change in color to brownish red and pale appearance in some parts the gills, swollen, filled with mucus, and the gill filaments are not neatly structured. On the other side, the mild gross lesion were shown in gills with a low degree of infestation, which only found of

excess mucus in the gills without any changes in color and structure. Changes in the anatomical pathology of the gills found are similar with the results of research by Sudaryatma and Eriawati (2012) which reported that the gills will have some gross lesion in anatomical pathology when infested by *Dactylogyrus* spp., which is characterized by blackish red gill color and inflammation.

The result of this study is similar with Mora *et al*. (2022) which showed that hyperplasia, secondary lamella fusion, telangiectasia, and vacuoles were found in the gill tissue which infested by *Dactylogyrus* spp. These different types of damage can be caused by several factors, such as the amount of parasite infestation, nutrition, length of time of infection, and individual immunity.

The damage that occurs based on these observations is damage that occurs gradually, which begins with hyperplasia, secondary lamella fusion, and vacuoles, then telangiectasia occurs. Hyperplasia in this study was found in the secondary lamella of the gills at low, medium, and high levels of infestation. According to Haqqawiy *et al*. (2013), hyperplasia can occur in secondary lamella and primary lamella. Hyperplasia in secondary lamella is caused by excessive epithelial cell division, while hyperplasia in primary lamella is caused by excessive chloride cell division. Priosoeryanto *et al*. (2010) suggest that hyperplasia occurs at low levels of irritation which is always accompanied by an increase in the number of mucous cells. Tissue damage in the form of hyperplasia results in the thickening of the epithelial tissue at the end of the filament which is commonly referred to as distal clubbing. In addition, it can also result in the thickening of epithelial tissue located near the base of the lamella or referred to as the basal lamella.

According to Yolanda *et al*. (2017), the increase in mucus during hyperplasia is an effort of self-protection mechanism against foreign materials that infest and directly contact the gills. Then the mucus produced has a negative impact on the fish respiration system, where the mucus can cover the surface of the gill lamella so that the exchange of oxygen and carbon dioxide is inhibited. If hyperplasia occurs continuously, the space between secondary lamellae will also be filled continuously with new cells, causing attachment between secondary lamellae or referred to as secondary lamella fusion (Sugiantari *et al*., 2022).

Secondary lamella fusion in this study was found at moderate and severe degree of infestation. Secondary lamella fusion indicates a lack of gas secondary lamella diffusion efficiency due to hyperplasia in fish gill epithelial tissue and fusion of secondary lamella (Yolanda *et al*., 2017). According to Juanda *et al*. (2022), secondary lamella fusion in secondary lamella cause incomplete functioning of the lamella because the lacunae containing red blood cells are covered by damaged secondary lamella epithelial cells. Secondary lamella fusion is also caused by the reduced elasticity of epithelial cells in supporting organelles in the cell, causing a rupture in the lamella.

Sukarni *et al*. (2012) stated that the occurrence of secondary lamella fusion results in disruption in the process of oxygen uptake. If secondary lamella fusion occurs continuously and gets worse, it will cause vacuoles. Vacuoles in this study were found on primary lamellae in a high infestation. Vacuoles are characterized by blank spaces that are not stained by the hematoxylin-eosin dye. This vacuole occurs due to fatty degeneration caused by the accumulation of excess fat in the cytoplasm (Sugiantari *et al*., 2022). According to Sukarni *et al*. (2012), vacuoles which are empty spaces in the primary lamella occur due to necrosis in a cell or group of cells. Cells that experience necrosis due to hyperplasia and lamella fusion can be recognized by the shape of the cell nucleus which is reduced or pyknotic, enlarged, and lost or karyolysis. In this process, the cytoplasm is also karyolysis and lost. Therefore, these tissue parts can not absorb the hematoxylin-eosin dye given in the preparation process.

Further damage caused by sustained vacuolation is telangiectasia. The results showed the presence of telangiectasia between the primary lamellae, which is at a high level of infestation. According to Lestari *et al*. (2018), telangiectasia appears due to the

occurrence of other tissue damage such as hyperplasia, and fusion. According to Lestari *et al*. (2018), telangiectasia appears due to the occurrence of other tissue damage such as hyperplasia, fusion, and vacuoles that indicate necrosis which causes one part to experience temporary swelling and the other part to shrink, resulting in the narrowing of blood vessels. This causes blood to accumulate in one part, forming a round balloon-like shape.

Conclusions

Based on the results of the study, it can be concluded that in common barb fish infested with *Dactylogyrus* there are changes in anatomical and histopathological pathology. Changes in anatomical pathology of the gills in the form of changes in color to red-brown or blackish red with some parts looking pale, filled with mucus, swelling, and unstructured neat gill filaments. Histopathology of the gills is indicated by damage in the form of hyperplasia, secondary lamella fusion, vacuoles to telangiectasia.

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